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Review

Advances in nanotechnology-based delivery systems for EGFR tyrosine kinases inhibitors in cancer therapy

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ABSTRACT

Oral tyrosine kinase inhibitors (TKIs) against epidermal growth factor receptor (EGFR) family have been introduced into the clinic to treat human malignancies for decades. Despite superior properties of EGFR-TKIs as small molecule targeted drugs, their applications are still restricted due to their low solubility, capricious oral bioavailability, large requirement of daily dose, high binding tendency to plasma albumin and initial/acquired drug resistance. Nanotechnology is a promising tool to improve efficacy of these drugs. Through non-oral routes. Various nanotechnology-based delivery approaches have been developed for providing efficient delivery of EGFR-TKIs with a better pharmacokinetic profile and tissue-targeting ability. This review aims to indicate the advantage of nanocarriers for EGFR-TKIs delivery.

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1. Introduction

The epidermal growth factor receptor (EGFR) family of receptor tyrosine kinases (TKs) has four homologous receptors including EGFR (ErbB1/HER-1), ErbB2 (HER-2), ErbB3 (HER-3) and ErbB4 (HER-4), and these membrane receptors have intracellular domains with TK activity. The ErbB proteins function through either homo- or hetero-dimerization. Following dimerization, specific tyrosine residues can autophosphorylate, ultimately resulting in the activation of downstream signaling pathways [1,2]. Overexpression and activating mutations of EGFR family receptors, specifically EGFR and HER2,

are associated with uncontrolled cell division of many human epithelial malignancies, including non-small cell lung cancer (NSCLC), breast cancer (BC), colorectal cancer, and pancreatic cancer [3]. For instance, an activating EGFR mutation has been observed in 20%–40% of Asian patients and up to 15% of Caucasian patients with NSCLC [4].

Therefore, the inhibition of EGFR family, especially EGFR and HER2, is an important therapeutic approach in the field of cancer therapy [5]. TKIs, mostly derived from quinazoline, can block the magnesium-ATP-binding site of the intracellular tyrosine kinase domain and *in vitro* studies confirmed their ability to disrupt tyrosine-kinase activity followed by the reduced activation of intracellular downstream signaling.

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In clinic small molecule EGFR-TKIs have shown the significant therapeutic efficacy against several cancers, which led to them as one kind of the most investigated small molecule inhibitors in cancer treatment [6–10].

To date, the U.S. Food and Drug Administration (FDA) has approved some TKIs targeting the EGFR family as tumor therapeutic agents and all the EGFR-TKIs available on the market are film-coated tablets for oral administration. However, the limited oral bioavailability associated with poor aqueous solubility, permeability, and extensive plasma protein binding capacity leads to restricted therapeutic efficacy [11,12]. Since EGFR signaling pathway also plays an essential role in proliferation, differentiation, migration, and apoptosis of normal cells, such as epithelial, mesenchymal, and neuronal cells, traditional oral delivery of EGFR-TKIs is accompanied by a series of side effects including rash, erythema, diarrhea, gastrointestinal (GI) perforations, ocular lesions, and hematological disorders [13,14]. Lipophilicity also mediates the low targeting efficiency of agents, promoting binding to unwanted targets [15]. Specially, Skin contains appreciable levels of EGFR and its inhibition may lead to skin lesions. Severe rash limits the anti-cancer treatment with a higher drug dosage. Additionally, for patients with GI dysfunction, like active peptic ulcer, inability to take oral medication, or prior gastrointestinal surgeries, the development and evaluation of different formulations of EGFR-TKIs are valuable.

In clinic, despite a significant initial response, the development of acquired resistance in most of the patients also limits the long-term efficacy of TKI therapy [16,17]. With great efforts made on the development of new generations of EGFR-TKIs, multi-agent combination therapy that impacts on multiple signaling pathways is also a promising approach. It has been confirmed that by affecting different signaling pathways in cancer cells, combination therapies can provide synergistic anticancer effects, overcome mechanisms of drug resistance and minimize side effects.

To date, appropriate drug combinations have brought significant benefits to cancer patients in clinic. Due to different pharmacokinetics and biodistribution of drugs and dissimilar rates of metabolism in the body, the optimized dose ratio is hard to be maintained. One of the grand challenges of multi-agent administration is how to reach sufficient concentration with a correct ratio of drugs in the target tissues.

The nano-technology based drug delivery system (DDS) is capable of bringing hope to solve the problems mentioned above. Efficient EGFR-TKIs nanoscale delivery systems not only help to solve the fundamental shortcomings such as poor solubility and rapid degradation but also minimize the side effects via the selective accumulation at tumor tissues [18–20]. Leaky and tortuous vasculature with poor or absent lymphatic drainage is one of the characteristic features of solid tumor tissues. Nanosized carriers can pass through the leaking blood vessels and accumulation in tumor tissues with the enhanced permeability and retention (EPR) effect, so-called as “passive” targeting [21]. Meanwhile, via specific coatings, better pharmacokinetics, internalization, and targeting of nanocarriers could be achieved. Nanocarriers are also identified as excellent delivery platforms which can deliver combined agents with correct ratio and well-designed release sequence to achieve the synergistic effects [22].

This review aims to discuss the nanotechnology-based DDS used for EGFR-TKIs delivery in cancer therapy. At the same time, we summarized antitumor mechanisms and clinical status of the FDA approved EGFR-TKIs for different types of cancer.

2. Clinical status of EGFR-TKIs

Since aberrant activation of EGFR signaling pathway is associated with development of variety of malignancies, numerous anti-tumor agents that primarily target EGFR family are currently at different stages of clinical development. This section provides a summary about the functional mechanisms and clinical states of the four most commonly used EGFR-TKIs approved by FDA for the therapy of solid tumors for more than 3 years. All the key properties are outlined in Table 1.

2.1. Gefitinib

As orally active 4-anilino-quinazolines, gefitinib (GEF) is able to effectively and reversibly target the ATP-binding pocket of the TK domain of EGFR.

GEF originally received accelerated approval of FDA in 2003 as a monotherapy for the treatment of patients with advanced or metastatic NSCLC after a failure of both docetaxel and platinum-based chemotherapies [23]. In subsequent clinical trials, however, no evidence was observed for a correlation between the extent of expression of EGFR and patient response to GEF. FDA updated the approval and restricted the use of patients in 2005. Screening of high responders conducted to the identification of sensitivity mutations in the TK domains of the genes for EGFR: exon-19 deletions or exon-21 (L858R) substitution mutations [24–26]. Ultimately, in 2015, the FDA approved GEF for the first-line treatment of patients with metastatic NSCLC bearing such mutations

In terms of safety, the most common adverse reactions were diarrhea skin reactions, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) increase. Approximately 5% of patients treated with discontinued treatment due to adverse reactions. Bone pain, dyspnea, pulmonary toxicity and stomatitis are observed occasionally [27].

2.2. Erlotinib

Erlotinib (ETB) is another ATP-competitive quinazoline derivative that works by binding to EGFR-TK in the same manner as GEF.

In 2004, ETB initially received FDA approval as monotherapy for the treatment of progressed NSCLC after the failure of at least one prior chemotherapy. In another Phase III trial, the combination of erlotinib (100 mg/day) with gemcitabine (1000 mg/m², weekly) significantly prolonged survival, especially progression-free survival (PFS), in patients with advanced pancreatic cancer [8]. This pivotal clinical trial led to the FDA-approval of erlotinib combined with gemcitabine in locally advanced and metastatic pancreatic cancer in 2005. Experimental results of several preclinical and clinical studies further demonstrated the association between EGFR mutations and response to ETB [28,29]. With this background, in 2013, ETB received approval by FDA for use as

Table 1 – Properties of FDA-approved small molecule EGFR tyrosine kinase inhibitors.

Name	Targets	The partition coefficient (log P)	Clinical dose	The FDA-approved indications (year)	Most common adverse effects
Gefitinib	EGFR	4.50	250 mg once daily	Locally advanced metastatic NSCLC cancer after failure of both platinum-based and docetaxel chemotherapies (2003)	Proteinuria, diarrhea, ALT increased, decreased appetite, AST increased, and skin reactions
Erlotinib	EGFR	3.14	150 mg once daily	NSCLC as a monotherapy after failure of at least one prior chemotherapy (2004) Advanced pancreatic cancer in combination with gemcitabine for patients who have not received previous chemotherapy (2005)	Skin rash, diarrhea, mucositis, hyperbilirubinemia, neutropenia, and anemia
Lapatinib	EGFR/ErbB2	4.97	1250 mg once day	Metastatic breast cancer in combination with capecitabine whose tumors overexpress HER2 and have received prior therapy, including an anthracycline, a taxane, and trastuzumab (2006)	Diarrhea and palmar-plantar erythrodysesthesia
Afatinib	EGFR/ErbB2/ErbB4	3.97	40 mg once daily	First-line treatment of NSCLC with exon-19 deletions or the exon-21 L858R mutation. (2013)	Diarrhea, vomiting, dyspnea, fatigue, and hypokalemia

a first-line treatment of NSCLC in patients whose tumors harbor EGFR exon-19 in-frame deletions or exon-21 (L858R) mutations.

As with GEF, skin reactions and diarrhea were the main adverse events found to be associated with ETB treatment. Mucositis, anemia, neutropenia, hyperbilirubinemia, and elevated ALT have also been reported. It uncommonly produces severe skin blistering and GI perforation in which case the drug must be permanently discontinued.

2.3. Lapatinib

Approximately 15%–20% of breast cancers overexpress HER2, classified as HER2-positive subgroup. HER2- positive BC cells tend to be more aggressive due to abnormal cell growth mediated via the overexpression of the HER2 protein [30,31]. One of critical therapeutic approaches in HER2-positive BC is inhibiting EGFR/HER2 with receptor-targeted TKIs [32].

Lapatinib (LAPA), a member of the 4-anilino-quinazoline family of TKIs, contains side chains which are different from those of GEF or ETB. It can potently and reversibly target and binds to the intracellular TK domains of EGFR and HER2 receptors. Therefore, in 2007, lapatinib was approved by the FDA as a combination with capecitabine for the treatment of advanced HER2 positive BC patients who have received prior therapy including trastuzumab and other anticancer agents. Subsequently, it was also approved by the FDA in combination with letrozole for the treatment of postmenopausal women with hormone receptor-positive advanced BC overexpressing the HER2 for whom hormonal therapy suggested [33]. A Phase III trial of 399 patients with advanced HER2 positive BC reported that compared to capecitabine alone group, combination group of lapatinib and capecitabine can prolong the median time to tumor progression (TTP) of patients [34].

Diarrhea and skin rash are the most common adverse effects attributed to the large requirement of daily oral dose

2.4. Afatinib

In clinic, most of the responders to GEF/ETB will inevitably develop acquired resistance with one or two years, and the most frequent resistance mechanism is the emergence of T790M mutation in the EGFR- tyrosine kinase domain [35,36]. With this background, irreversibly, covalently second-generation EGFR-TKIs including afatinib (AFT) were developed.

AFT is an oral aniline-quinazoline derivative with a reactive acrylamide group. It can irreversibly inhibit EGFR-family autophosphorylation followed by downregulation of EGFR signal transduction via covalently binding to EGFR, HER2, and HER4. AFT is the first irreversible EGFR-TKIs approved by FDA for NSCLC treatment.

According to the results of two open-label, randomized, phase III trials, AFT (40 mg/d) can bring prolonged PFS to advanced NSCLC patient with EGFR mutations compared with traditional chemotherapy [37,38]. Therefore, in 2013, oral AFT (Gilotrif™) received approval of FDA for the first-line treatment of patients with locally advanced or metastatic NSCLC who have proven EGFR mutations. The recommended dosage is 40 mg daily, and treatment should be continued until AFT is no longer tolerated or until disease progression.

The severity of common adverse events such as rash, erythema and diarrhea were found to be dose-dependent during AFT treatment. Cases of interstitial pneumonia, fulminating liver disease, severe skin blistering were also reported in a small percentage of patients, with some of these causing death.

3. Nanotechnology strategies for EGFR-TKIs delivery

3.1. Liposomes

The inherent advantages of liposomes, such as non-immunogenicity, biocompatibility, high drug loading capacity and controllable release manner, have promoted their widespread use as drug carriers in tumor therapeutics [39]. They are composed of single or multiple lipid bilayers encapsulating an aqueous space and lipid bilayers which mainly consist of cholesterol (CHOL) and phospholipids molecules [40].

The drug can be localized in lipid membrane or the internal aqueous core of liposome, depending on its lipophilicity [41]. Due to the membrane structure is similar to the cell membranes, which can facilitate cellular uptake of drugs in them, the liposomes are regarded as an ideal drug-carrier system to promising approach to improve the agents' biodistribution.

Liposomes are divided in three subtypes, (1) conventional type: it consists of the lipid bilayer and an aqueous core (2) PEGylated type: polyethylene glycol (PEG) is attached to the surface of liposomes to extend the blood circulation time by avoiding them from recognition and uptake of the reticuloendothelial system (RES), and (3) ligand-targeted type: ligands, including antibodies and peptides, are linked to the surface of the liposomes to obtain active targeting effect to specific cells.

Numerous research groups have developed several EGFR-TKIs loaded liposomal nanocarriers.

A PEGylated ETB liposome, with an entrapment efficiency (EE) of $85.3\% \pm 1.8\%$, mean size of 102.4 ± 3.1 nm and zeta potential of -16 mV, was composed of PEG-1,2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE), CHOL, Soybean phosphatidylcholine (SPC) at the ratio of 3:10:22 [42]. The cytotoxicity of blank liposomes, free-ETB and two formulations (with or without PEGylated ETB loaded liposomes) were examined *in vitro* studies. The results revealed that the blank liposomes had negligible cellular cytotoxicity. At the same incubation condition, liposomal ETB significantly increased the cytotoxicity against A549 NSCLC cells, compared with free ETB, which should contribute to the enhanced intracellular drug accumulation by the transportation of liposomes. *In vivo*, PEGylation can greatly change the pharmacokinetic behavior and improve drug bioavailability. As compared to conventional liposomes and free ETB solution, the PEGylated formulation showed longer terminal half-life, lower clearance rates and higher area under the curve $0-\infty$ values in pharmacokinetics analysis.

The tumor microenvironment is characterized by hypoxia, lower pH, high glutathione (GSH) levels, etc. which can be adopted as triggers to cause a release of cargo molecules from the system [43]. Through adding a kind of pH-sensitive phospholipid, cholesteryl hemisuccinate (CHEMS), Alanood et al. synthesized pH-sensitive liposomes to delivery AFT in lung cancer cells [44]. Owing to PH-responsive release and negative values of zeta potential, the anticancer efficiency of this PH-sensitive liposome was significantly higher compared with conventional and cationic liposomes containing AFT *in vitro*.

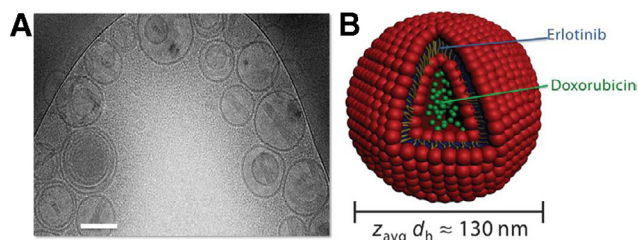


Fig. 1 – Characterization of the combination therapeutic-loaded liposomal system. (A) Cryogenic transmission electron micrograph of dual drug-loaded liposomes. Scale bar, 100 nm. (B) Schematic of dual loading of a small-molecule inhibitor (ETB, blue) into the hydrophobic, vesicular wall compartment and of a cytotoxic agent (DOX, green) into the aqueous, hydrophilic interior.

Encapsulating EGFR-TKIs with other anticancer agents is another formulation strategy used in cancer therapeutics. Because the synergistic effect could improve the anticancer effects and reduce the cytotoxicity toward normal cells by affecting multiple signaling pathways and releasing the drugs either simultaneously or in a predetermined sequence at target sites.

It has been proved that via prolonged EGFR inhibition, cancer cells could re-acquire a working apoptosis pathway responsive to DNA damage [45]. In detail, researchers found that EGFR inhibition dramatically sensitizes a subset of breast cancer cells to DNA damage if the drugs are given sequentially but not simultaneously. Furthermore, transcriptional, proteomic, and computational analyses of signaling networks and phenotypes in drug-treated cells revealed the enhanced treatment efficacy results via dynamic network re-wiring of an oncogenic signature maintained by active EGFR signaling to unmask an apoptotic process which involves activation of caspase-8. The increased sensitivity to chemotherapeutic agents observed required sustained inhibition of EGFR because the phenotype did not result from a rapid direct inhibition of the oncogene, but rather from modulation of an oncogene driven transcriptional network as indicated schematically. Distinct examples of multidrug delivery systems containing EGFR-TKIs for cancer therapy are depicted in Table 2.

Morton et al. [46] successfully designed a liposomal DDS which not only co-deliver drugs with different properties but also release them with the desired time sequence. By entrapping ETB in the hydrophobic lipid membrane and loading doxorubicin (DOX) into the hydrophilic compartment, ETB was released before DOX (Fig. 1). In brief, the liposomes containing DOX and ETB were successfully fabricated via a lipid film hydration method. Lipid vesicles were formed after hydration in an acidic citric acid buffer under sonication and high heat in the presence of the hydrophobic ETB. Subsequent pH-driven loading of DOX in the interior of the vesicles formed the final dual-drug liposomal system. Dynamic light scattering showed that the liposomes prepared in this two-step drug loading process were of uniform size on the basis of their polydispersity index (PDI), and similar results were observed via transmission electron microscope (TEM). *In vitro*, the sensitization effect was mediated by prolonged EGFR inhibition induced internal rewiring of apoptotic signaling networks.

Table 2 – Examples of nano-based multidrug delivery systems containing EGFR-TKIs for cancer therapy.

Nanocarrier type	Drug combinations	Delivery approach	Indications	Observations	References
Liposomes	ETB and DOX	Folate to folate receptor	NSCLC	The pretreatment of ETB could enhance rewiring of apoptotic signaling networks of cancer cells, leading to improved cytotoxicity of DOX.	[46]
Liposomes	ETB and PFOB	Oligonucleotide Apt to EGFR	NSCLC	This approach, oxygen and ETB co-delivery, was able to ameliorate the solid tumor microenvironment, overcome Hypoxia-triggered drug resistance and thus improve anti-cancer effect <i>in vitro</i> and <i>in vivo</i> .	[48]
MSNs	GEF and DOX	Cetuximab to EGFR	EGFR-resistant NSCLC	The co-delivery MSNs overcame EGFR-TKI resistance NSCLC with superior targeting ability mediated by cetuximab.	[69]
MSNs coated by PH-responsive lipid film	ETB and DOX	Passive	NSCLC	The charge conversion at tumor pH facilitated the cellular uptake of MSNs by the cancer cells and sequentially release of drugs further enhanced antitumor activity.	[71]
CS-NPs	GEF and CQ	Passive	Drug resistant TNBC	CQ as an inhibitor of autophagic lysosome formation could overcome autophagy in the drug resistant BC cells and effectively reversing GEF-resistance.	[94]
PMs	CYP and GEF	Passive	Pancreatic cancer	GEF/CYP co-loaded PMs showed a synergistic effect against pancreatic cancer cells mainly attributed to obstruction of sonic hedgehog and EGFR signaling pathways.	[103]
PEG-PBC micelles	LAPA and DOX	Passive	DOX-resistant BC	The use of lapatinib not only as a dual TKI but also as an adjuvant, inhibiting efflux of P-gp, sensitized MDR cancer cells to DOX.	[109]
PEG-PCD micelles	LAPA and PTX	Passive	MDR prostate cancer	LAPA can minimize the efflux and enhance the intracellular accumulation of chemotherapeutic agents in MDR prostate cancer	[104]
PEG-PLA micelles	LAPA and PTX	Passive	HER2-positive BC	PPM-LP exhibited significantly stronger cytotoxicity ($p < 0.05$) to SKBr-3 cells (HER-2 positive) and showed almost no significantly different cytotoxicity ($p > 0.05$) to MDA-MB-231 cells (HER-2 negative) as compared with PPM PTX	[113]
Polymeric implants	LAPA and PTX	Passive	TNBC	This localized co-delivery system revealed steady drug accumulation in tumor sites and good synergistic effects between LAPA and PTX against TNBC	[114]
Nanovesicles	GEF and DOX	Passive	NSCLC	Tumor tissue or cell specific drug release mediated by reduction and pH dual-responsive nanovesicles further improved efficacy of combination therapy in lung cancer.	[118]
Self-assembled NPs	EBT and quercetin	Passive	NSCLC	Through combining both ETB and quercetin in one system could inhibit the phosphorylation at both upstream and downstream of EGFR signaling pathways and thereby more efficiently interrupt the EGFR signaling and eventually prevent the growth of EGFR-expressing tumors.	[121]

In order to further improve the therapeutic effect of EGFR-TKIs, great efforts have been made to develop innovative liposomes with targeting ability. Li et al. [47]. prepared liposomes anchored with anti-EGFR aptamer (Apt)-conjugated chitosan (Cs) and subsequently loaded ETB into these liposomal complexes (Apt-CL-E). After adjusting the ratio of lecithin, CHOL and CS, the drug loading (DL) of ETB has been optimized to 8% with nearly 100% EE and relatively stable mean particle size of 185 nm. *in vitro*, Apt-CL-E had specific cell recognition, increased cellular uptake and better antiproliferative effect in H1975 (EGFR-TKI-resistant) NSCLC cell lines, as compared with regular ETB-loaded liposomes without anti-EGFR aptamer conjugated. The results *in vitro* demonstrated that

aptamer played an important role in increasing ETB intracellular accumulation and inducing more cell cycle arrest and apoptosis to overcome acquired EGFR-TKI resistance in NSCLC.

A similar attempt has been made for targeting delivery oxygen and ETB by Apt-modified liposomal complexes to reverse hypoxia-induced drug resistance [48] (Fig. 2). Novel multifunctional liposomes anchored with anti-EGFR Apt-conjugated CS (ACLEP) were formulated to co-delivery ETB and an artificial blood substitute, perfluorooctyl bromide (PFOB), for reversing hypoxia-induced drug resistance. Using thin film hydration method, ACLEP exhibited suitable particle sizes (184.8 ± 5.87 nm), narrow PDI (0.200 ± 0.012), applica-

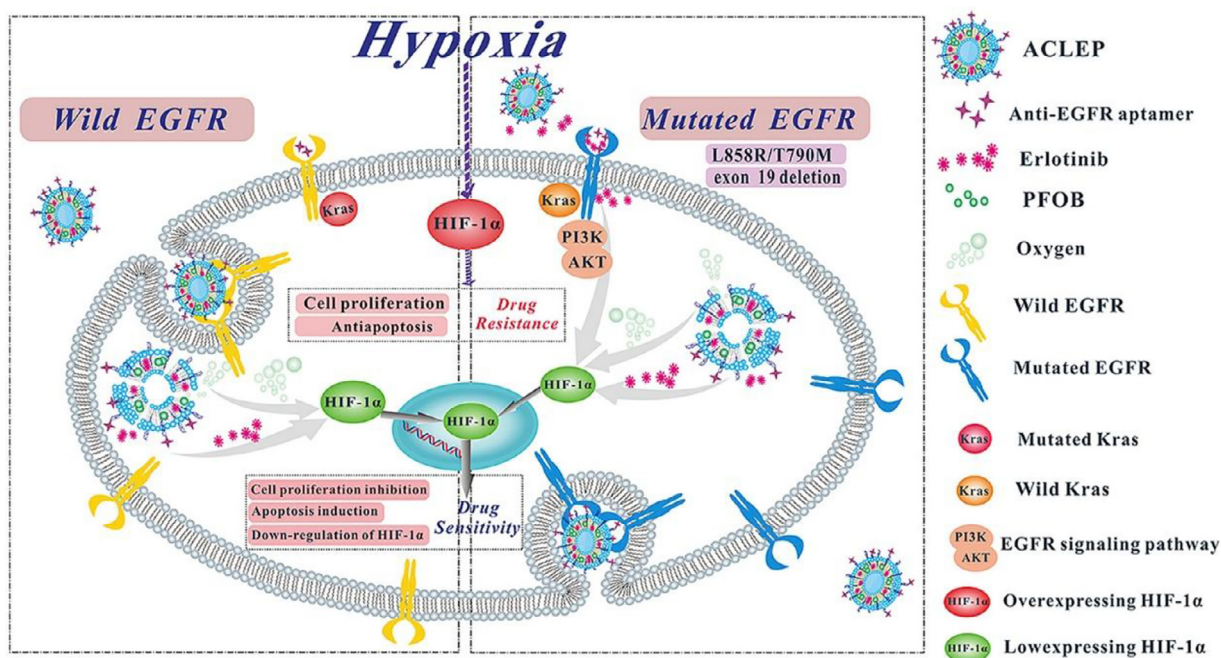


Fig. 2 – Schematic illustration of the mechanisms of action of ACLEP to overcome hypoxia-triggered erlotinib resistance. ACLEP could achieve oxygen and drug co-delivery and regulate the expression of HIF-1 α in hypoxic microenvironment in both EGFR-wild and EGFR-mutated NSCLC.

ble zeta potentials (33.55 ± 0.64 mV), superior oxygen content (0.406 ± 0.14 mg/ml) and acceptable biostability.

Hypoxia-inducible factor-1 α (HIF-1 α), a subunit of HIF-1, could be responsively up-regulated when tumor cells are exposed to a hypoxic environment, promoting angiogenesis and tumor growth [49,50]. Previous investigations have reported that hypoxia significantly increased the population of tumor cells resistant to EGFR-TKIs in NSCLC with up-regulated HIF-1 α and EGFR expression [51,52]. The 50% inhibitory concentration (IC₅₀) values of ETB against A549, PC-9 and H1975 NSCLC cell lines were 5.65-, 6.12- and 3.28-fold increase in hypoxic condition. *in vitro* studies, compared to ordinary liposomal ETB this novel formulation, ACLEP, significantly inhibited cell proliferation, induced apoptosis, and down-regulated the expression of the protein EGFR, p-EGFR and HIF-1 α in these NSCLC cells. Authors suggested that the enhanced anti-tumor efficacy was mainly associated with specific binding to overexpressing EGFR receptor of NSCLC cells guided by the anti-EGFR Apt and thereby efficiently delivery ETB and oxygen into hypoxic cells.

Manifested anti-tumor efficacy of ACLEP was evidenced by reduced tumor growth in A549-bearing mice. The *in vitro* and *in vivo* results consistently evidenced that the drug and oxygen co-delivery approach could be an effective method to modulate the solid tumor microenvironment and improve the efficacy of chemotherapy.

3.2. Protein-based nanocarriers

Protein-based DDS have been exploited and utilized for decades due to their biocompatible, non-immunogenic and biodegradable properties coupled with low toxicity [53,54].

In biological systems ferritin (FT) is used to store iron, and apoferritin (apo-FT) is formed when the iron atoms are removed from FT. FT/apo-FT protein cage is composed of 24 subunits which assemble into a 12 nm diameter cage with an internal 8 nm hollow span. This protein cage also has 14 channels to allow the exchange of contents between the interior and exterior environments [55,56]. In many malignant tumor cells, such as brain and breast cancer cells, transferrin receptors (TfR) highly express, which can mediate membrane-specific endocytosis. Also, FT and apo-FT can disassemble into subunits at low pH (< 2) allowing the release of cargo, and reassemble at higher pH (> 8.5) [57]. Due to its particular structure, reversible assembly/disassembly behavior and targeting ability, FT/ apo-FT has been studied as an interesting nanocarrier.

In a study by Anchalam et al. [58], ten molecules of GEF were passively loaded in each apo-FT molecule with a small particle size of 12 nm. This nanocarrier was utilized to target TfR positive SKBR3 cell lines. Using the fluorescent property of GEF, they observed facilitated intracellular drug accumulation in the apo-FT-encapsulated-GEF group from confocal microscopy and flow cytometry. *in vitro*, the cytotoxicity of apo-FT-encapsulated-GEF was much higher than that of GEF alone, which resulted from increased endocytosis and PH-responsive controlled drug release.

In recent years, albumin also attracts substantial interest as an attractive carrier due to its properties including high stability, nontoxicity, biocompatibility and biodegradability. It can also be preferentially taken up by tumor cells as a nutrient. In addition, the existence of charged functional groups, like amino and carboxylic groups, offers albumin with chances to interact with a wide variety of therapeutic agents [59–61].

Wan et al. [62] prepared LAPA-loaded human serum albumin (HSA) nanoparticles (LHNP) with a narrow size distribution of 140 nm and good EE of nearly 85% for intravenous administration by employing nanoparticle albumin-bound technology (Nab™). Nab™ developed by American Bioscience, Inc. is an innovative technique for preparing hydrophobic drugs loaded albumin nanoparticles (NPs) by passing through the aqueous mixture of therapeutic drug molecules and albumin under high pressure through a jet [63]. The LHNP displayed enhanced stimulation of apoptosis in 4T1 monolayer cells and facilitated penetration and inhibitory efficacy in tumor spheroids relative to LAPA solution. Intravenous injection of LHNP in mice led to 16-fold increased tumor accumulation compared to Tykerb™, the commercial tablets of LAPA for oral administration, and prevented lung metastasis at one-tenth dose of Tykerb™.

Noorani and co-workers developed ETB-loaded albumin nanoparticles (E-ANPs), by desolvation method with a mixed solvent followed by thermal cross-linking for stabilization, which is one of the best approaches to achieve stable biodegradable structure [64,65]. The *in vitro* cytotoxicity of E-ANPs against ASPC-1 and PANC-1 pancreatic cancer was higher than ETB solution at 2.9 and 3.0-fold, respectively. Authors suggested that albumin encapsulation boosted the specific endocytic uptake of ETB, which thereby promoted antiproliferative effects.

E-ANPs seem to be a promising therapeutic agent for pancreatic cancer, and *in vivo* tests for further evaluation are needed.

3.3. Inorganic nanocarriers

In past decades, inorganic nanocarriers such as silica, gold, graphene and carbon nanotubes have been exploited as drug delivery vehicles, on account of their versatile physicochemical properties including biocompatibility, low cytotoxicity, readily availability, easily functionalization and ability to accumulate in cancer cells without P-Glycoprotein (P-gp) recognition, causing increased intracellular concentration of drugs [66,67]. Though their delivery efficiency appears to be lower than that of cationic carriers and viral at present, inorganic nanocarrier with modified surface employing bio-functional molecules like antibodies, aptamers and proteins can effectively reduce the gap [43].

Silica exists in many forms such as silica particle, nanotube, hollow silica particle, mesoporous silica nanoparticle (MSN), and hollow mesoporous silica nanoparticle (HMSN) of which the latter two are more promising in the field of nanodDS. Large surface areas, high pore volume, high chemical stability, high drug-loading capability and easily functionalized surface are the main advantages of HMSN/MSN in the field of nanocarriers [68].

Wang et al. [69] selected cetuximab, the anti-EGFR monoclonal antibody, as targeting ligand to maximum specific uptake of drug loaded MSN by EGFR overexpressing tumor cells. Subsequently, they developed cetuximab-modified MSNs (CET-capped MSNs) through the cross-linking of disulfide bond. This unique MSNs were designed as drug delivery platforms to simultaneously, specifically release DOX and GEF to targeting tumor cells with high EGFR expression. The re-

searchers reported that in PC9 cells, a GEF resistant NSCLC cell line, CET-capped MSNs facilitated the endocytosis and reached high intracellular accumulation in a time-dependent manner, which was confirmed through flow cytometry analyses. *in vitro* and *in vivo*, CET-capped GEF/DOX MSN was showed a superior inhibitory effect for PC9 NSCLC cells relative to ordinary co-loaded MSNs. All the findings corroborate that this unique DDS might serve as a great strategy to overcome EGFR-TKI resistance and be a potential drug candidate for the management of EGFR-mutant lung cancer. After being endocytosed, CET-capped MSN could release loaded drugs via the cleavage of disulfide bond through the interaction with the high level of GSH in cytoplasm of cancer cells.

As compared with the approach of active targeting ligand modification, the nano-systems using pH value of tumor microenvironment for improved cellular internalization is easy to prepare and can be applied for the treatment of all kinds of tumor [70].

With this background, He et al. [71] successfully developed pH-responsive charge-reversal MSNs to delivery synergistic ETB and DOX combination with sequential release manner for the treatment of NSCLC (Fig. 3). Besides, the study by Lee et al. emphasized the importance of a fixed period of time between the administration of each drug for maximizing the synergistic effect of combination chemotherapy [45].

This pH-sensitive charge-reversal lipid film consisted of SPC/ 1, 5-Dioctadecyl-L-glutamyl 2-histidyl-hexahydrobenzoic acid (HHG2C18) /Chol at a weight ratio of 3.75:1.25:1, which were able to reverse surface zeta potential from negative to positive at tumor acidic extracellular microenvironment, then facilitate the internalization for the targeted cancer cells. In this platform, ETB was released faster than DOX intracellularly, because the lipid membrane containing ETB was coated on the surface of DOX-loaded MSN. *In vivo* data showed that this ETB/DOX co-loaded pH-responsive charge-reversal MSNs inhibited tumor growth effectively with negligible systemic toxicity.

Gold nanoparticles (AuNPs), a kind of metallic nanoparticles, in sizes of 1–100 nm are extensively used for drug and gene delivery on account of its inertness, excellent biocompatibility and lower toxicity than other metal material. Besides, bioavailability, targeting ability and endocytosis of AuNP can be greatly improved with a functionalized surface [72–74]. In this context, one of the most commonly used compounds for modification of AuNPs is PEG which can be covalently bound to the surface atoms of AuNPs.

In a recent study, a DDS based on PEGylated AuNPs conjugated with AFT (PEG-AuNPs-A) was designed with a mean size of 41 nm, low polydispersity index PDI, zeta potential of –35 mV and high store stability [75]. Coelho et al. tested the drug activity against pancreatic cancer cells (S2-013) and NSCLC (A549) by the evaluation of *in vitro* cytotoxicity and further identify the mechanisms via confocal imaging and flow cytometry. The results showed that 5 and 20 times AFT alone are required than PEG-AuNPs-A to induce 50% cell survival in S2-013 and A549 cells with a higher cellular uptake of PEG-AuNPs-A observed by cancer cells. All their findings reinforced that uptake and cytotoxicity of AFT in pancreatic and lung cancer cells can be highly enhanced by conjugated with PEG-AuNPs.

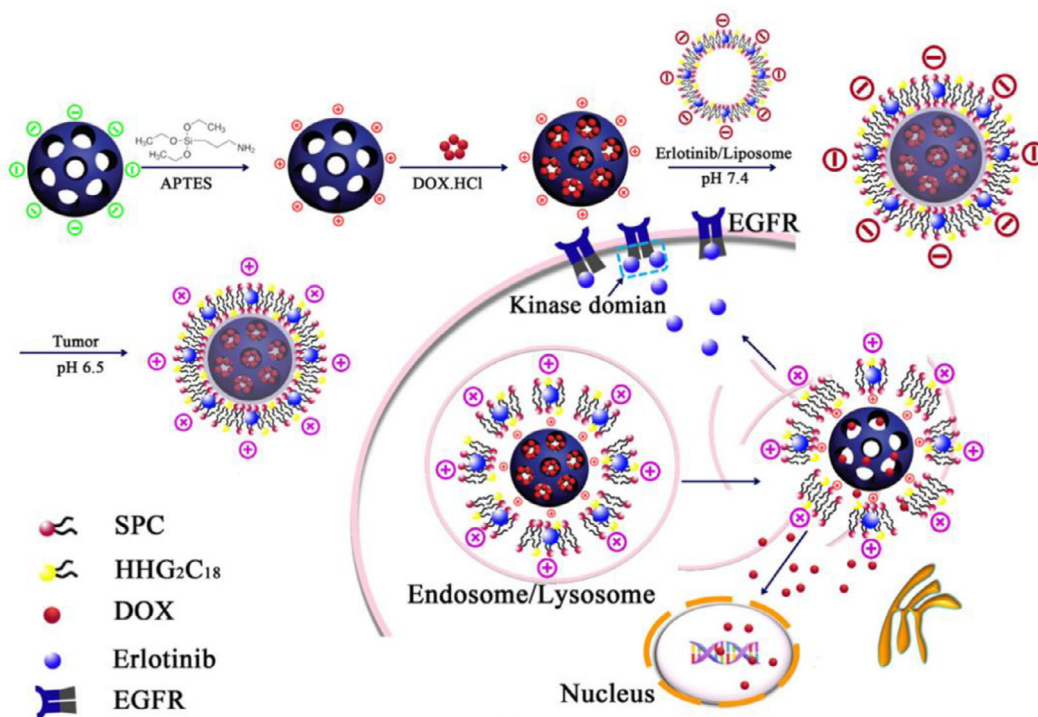


Fig. 3 – Schematic illustration of preparation of ETB/DOX combination co-delivery nanocarriers and synergistic therapy of ETB and DOX. After administration, MSNs were positively charged at extracellular environment leading to an easy internalization by tumor cells. As ETB was loaded in the exterior lipid bilayer and the controlled releaseability of MSN, after entering into tumor cells ETB released faster than DOX.

Smart stimuli-responsive DDS has provided a profound impact on preclinical and clinical applications. Of these systems, thermo-responsive DDS can achieve controllable drug release by applying heat whenever necessary. Poly(*N*-isopropylacrylamide) (PNIPAAm) regarding as thermo-sensitive polymers is generally used in the development of nanocarriers [76–78].

ETB encapsulated CS copolymer-gold hybrid (CGH) NPs as smart thermo-responsive nanocarriers was synthesized via autoreduction of auric cations [79]. In brief, oleic acid (OA) and (*N*-isopropylacrylamid) (NIPAAm) were copolymerized on the modified CS to obtain desired thermo-responsive copolymer and then CGH-NPs were formed through the reduction ability of amino functional groups of CS copolymer. ETB can be facilely loaded in CGH-NPs with the EE of 30% since PNIPAAm chains are hydrophobic. The ETB was released from the CGH-NPs in a thermo-responsive manner. Subsequently, flow cytometry analysis corroborated high cellular uptake (85.81%) of CGH-NPs by A549 lung cancer cells and the cytotoxicity evaluations proved (the) high anti-tumor activity of the ETB loaded CGH NPs with an excellent cytocompatibility.

3.4. Polymeric nanoparticles

Polymeric nanoparticles (NPs) can effectively provide more accumulation of drugs within the solid tumor tissues because of nanoscale induced EPR and greater adsorption to cells. They are also able to improve the pharmacodynamic and pharmacokinetic properties of various bioactive

molecules to overcome part of the limitations in traditional formulations [80].

Poly (D,L-lactic-co-glycolic acid) (PLGA) is a copolymer approved by the FDA for medical applications because it can undergo hydrolysis in the body and produce the biodegradable metabolite monomers, glycolic acid and lactic acid [81]. PLGA-NP is one of the excellent approaches for improving bioavailability and enhancing the treatment efficacy of EGFR-TKIs. A study in rat showed that ETB loaded PLGA-NPs enhanced the oral bioavailability and reduced subacute toxicity of ETB, and this can be attributed to the improved pharmacokinetics, sustained release and a longer residence time [82].

Recently, an original synthetic method to obtain ETB-loaded PLGA-NPs was developed by Vaidya et al. [83]. The authors suggested that by preparing PLGA-NPs with multiple emulsion solvent evaporation methods with water-soluble cyclodextrin complex, the entrapment of ETB in the NPs increased almost 3-fold than reported single emulsion method. The obtained PLGA-encapsulated ETB NPs (PLGA-ETB) showed a mean size of 210 ± 8 nm, DL of 5% and sustained release profile. Developed NPs also demonstrated improved efficacy against NSCLC cells in terms of increased apoptosis, low IC₅₀ values, and autophagy inhibition.

Polymeric NPs made from poly(ϵ -caprolactone)-poly(ethyleneglycol)-poly(ϵ -caprolactone) (PCEC) has also been utilized as a biocompatible nanocarrier to effectively delivery hydrophobic EGFR-TKIs. In the research by Xiao et al. [84], GEF-loaded PCEC (PCEC-GEF) NPs were developed via a

solid dispersion method, with an average size of 24 nm, zeta potential of -18 mV, DL of 9% and EE of 92%. This PCEC-GEF NPs could slowly release GEF and within 5 days 80% cargo molecules were released in controlled and sustained behavior. This might be the reason why PCEC-GEF has higher IC₅₀ against lung cancer cells (A549) *in vitro* after 24 and 48 incubation than GEF solution. *In vivo*, the experimental results showed that the intravenous injection of PCEC-GEF NPs led to a significantly longer delay on tumor growth, lower GEF-related side effects and an increased median survival time of approximately 23 d compared with GEF treated group. Subsequent flow cytometry and evaluation of the expression of Ki-67 and CD31 was conducted in order to further investigated the mechanism of anticancer action of PCEC-GEF NPs *in vivo*. The results suggested this GEF-NPs could effectively increase cellular apoptosis, reduce proliferation and inhibit angiogenesis of solid tumor in a xenograft mouse model induced by lung cancer cells.

Natural polymers such as chitosan, a hydrophilic polysaccharide, is a natural polymer [85]. Similar to other biopolymers, chitosan has validated biodegradability, biocompatibility, non-immunogenicity, high mucoadhesion, fungistatic and antimicrobial activity [86,87].

Autophagy, a basic catabolic mechanism, involves cell degradation of dysfunctional or unnecessary cellular components through the actions of lysosomes. During the autophagic process, the autophagosome and lysosome were fused to develop autophagic lysosome, degrading deformed molecules in cytoplasm and organelles, and then recycling the degraded product to provide nutritive material for the survival of cells [88–90]. According to recent investigations, autophagy may take dominated part in promoting acquired drug resistance of cancer cells. Autophagy facilitates cancer cells to survive in the adverse environment by accelerating the growth of cancer cells and preventing antitumor agents from killing cells [91–93].

CS-NPs entrapping both GEF and chloroquine (CQ), a known inhibitor of autophagic lysosome formation, were fabricated by Zhao et al. and their ability to enhance the delivery of anticancer agents against acquired MDR tumor cells was validated [94]. Western blot analysis also confirmed that the ratio of LC3I to LC3II as an autophagosome marker was decreased and expression of caspase-3 protein as the main apoptosis relevant protein was elevated.

3.5. Polymeric micelles

Polymeric micelles (PMs), a fast-growing area in the field of drug delivery, are composed of hydrophilic and hydrophobic functional groups which can self-assemble in aqueous media when the concentration of amphiphilic copolymers exceeds critical micelle concentration (CMC) [95,96]. Also, micelles are the core-shell structure where the core is formed by the hydrophobic part and the shell is formed by the hydrophilic part. This structure is more stable than liposomes with a smaller size range and provides the possibility to simultaneously encapsulate multiple therapeutic agents with different physicochemical properties. Concomitantly, the PEGylated shell prevents the uptake of PMs by (the) reticuloendothelial system (RES). Drugs may passively be loaded or covalently attached

to the PMs depending on the preparation. These intrinsic and modifiable properties of PMs make them primely suited for drug delivery applications [97].

An injectable formulation, AFT-loaded PM, consisted of AFT/ MPEG-PCL / Mal-PEG-PCL at a ratio of 1:9:1 was successfully fabricated by film hydration method [98]. *In vitro*, the experimental findings indicated that AFT-encapsulated PMs showed more cytotoxicity than free AFT solution did. The improved pharmacokinetics, tissue-distribution as well as the enhanced anticancer activity of AFT-encapsulated PMs were confirmed with HER2-overexpressing HCT-15 cell line induced colon tumor model.

Fathi et al. [99] synthesized CS based micelles grafted with PNIPAm as a temperature-sensitive monomer and oleic acid as hydrophobic segments. The PMs were modified with folic acid and loaded with ETB for cancer-specific targeting drug delivery. Folate-(PNIPAAm-co-OA)-g-CS micelles were obtained through a self-assembly process resulting in stable core-shell nanostructure with a mean diameter of 100 nm and zeta potential of -10 mV. The ETB loaded PMs showed good EE of nearly 40%, excellent biocompatibility, and a temperature-responsive release profile. In cellular uptake and cytotoxicity assays *in vitro*, a specific targeting behavior and enhanced cytotoxic efficacy of ETB-loaded folate-(PNIPAAm-co-OA)-g-CS micelles were observed in the folate-positive OVCAR-3 ovarian cells, which indicated the potential application of this micellar system as an injectable formulation in targeting therapy of folate positive cancer cells.

Several evidences have indicated that aberrant activation and cross-talk in sonic Hedgehog and EGFR signaling pathways play major roles in pancreatic carcinogenesis, disease progression, metastasis, and chemoresistance [100–102]. However, the low aqueous solubility of GEF and cyclophamide (CYP), a hedgehog pathway inhibitor, greatly limited their effective clinical translations as a promising approach. To resolve this issue, Chitkara et al. co-loaded GEF and CYP into PEG-b-poly(carbonate-co-lactic acid) (PEG-b-p(CB-co-LA)) micelle efficiently using the nanoprecipitation method [103]. The micelles displayed uniformed spheres with an average diameter of 54 nm and low PDI. *In vitro*, compared with free GEF and CYP, GEF/CYP co-loaded PMs showed a synergistic effect against pancreatic cancer cells and specifically increased Caspase 3/7 activity, indicating apoptotic cell death, compared with free GEF and CYP. Also, this co-delivery micellar system decreased tumor growth rate in the xenograft mouse model induced by pancreatic cancer cells.

In clinic, the prolonged use of chemotherapeutic agents inevitably induces multiple-drug resistance (MDR) and eventually lead to therapy failures [104]. This is mainly because of the overexpression of MDR transporters, the ATP-binding cassette (ABC) superfamily, which can increase drug efflux and decrease drug accumulation in tumor cells [105,106]. To date, three subtypes of MDR efflux pumps in cancer have been characterized: P-glycoprotein (P-gp; MDR1), breast cancer resistance protein (BCRP) and multidrug resistance-associated protein 1 (MRP1). Researches have indicated that LAPA could inhibit the function of ABC transporters and thus sensitize MDR tumor cells to chemotherapeutic drugs [107,108].

With this background, Wang et al. [109] synthesized Poly(ethylene-glycol)-blockpoly(2-methyl-2-benzoxycarbony-

lpropylene carbonate) (PEG-PBC) polymers for fabricating LAPA and DOX co-loaded PMs by film dispersion method. The low critical micelle concentration (CMC) of PEG-PBC (1.5 mg/L) with an average diameter of 100 nm implied its high dynamic stability. MCF-7/ADR cells is a drug resistant BC cell line with overexpressed P-gp. *In vitro*, researchers observed almost no intracellular accumulation of DOX after 4 h incubation with free Dox solution in MCF-7/ADR cells. Cytotoxicity tests also demonstrated that this co-delivery system notably increased anticancer activity of DOX in drug resistant BC cell lines. Moreover, the DOX-LAPA-PMs showed a significant decrease in tumor growth compared to DOX mono-therapy in the xenograft mouse model.

Similarly, Li et al. [110] synthesized the di-block polymer, poly(ethylene glycol)-block-poly (2-methyl-2-carboxyl-propylene carbonate-graft-dodecanol) (PEG-PCD) to develop LAPA and PTX co-encapsulated PMs for the treatment of MDR prostate cancer. The obtained PMs showed a mean size of 60 nm with nearly 100% EE of both drugs. Results from experiments confirmed that polymer PEG-PCD efficiently delivered a combination of LAPA and PTX to prostate cancer cells *in vitro* and *in vivo* with negligible cellular and systemic toxicity. In addition, with the inhibitory effect of LAPA on P-gp function, the toxicity of PTX dramatically enhanced in P-gp overexpressing prostate cancer cells.

The overexpressed HER2 in BC is associated with poor prognosis and chemotherapy resistance, thus targeted therapy aiming at HER2 is regarded as an important strategy for BC treatment. Combining LAPA with other traditional chemotherapies such as PTX has revealed a higher antitumor efficacy in recent years, for example, two phase III studies were conducted to investigate the combination effect of LAPA plus PTX against HER2-positive metastatic BC, and ultimately the combination therapy offered a significant survival advantage over PTX mono-therapy [111,112].

Wei et al. designed a PMs to better simulate clinical administration, long-term release of LAPA and short-term release of PTX, against HER2-positive BC [113]. In order to enhance the incorporation of LAPA in micelles, researchers firstly synthesized LAPA-conjugated poly (ethylene glycol) (PEG) and poly (lactic acid) (PLA) (L-PEG-PLA). Subsequently, micelles were prepared by thin film hydration method, entrapping PTX during the process, with a high drug loading capacity. Findings of cytotoxicity studies, employing HER2-positive BC cell line (SKBr-3), exhibited the enhancement of anticancer efficacy of PTX with sustaining action of LAPA, as evidenced by the reduction in IC₅₀ value of co-delivery of PMs. Also, the results of apoptosis assay were consistent with findings of *in vitro* cytotoxicity, as increased apoptosis index was observed in cells in combined PMs

3.6. Polymeric implants

Polymeric implants, capable of encapsulating small molecule substances in a polymeric matrix, have exhibited notable potential for delivery of various bioactive molecules including therapeutic drugs, proteins, and gene with a controlled rate. Polymeric implants can protect encapsulated drugs from degradation and provide *in situ* treatment to specific anatomic sites with a continuous sustained release of drugs and mini-

mized systemic exposure [114,115]. LAPA is characterized by poor oral bioavailability and high binding tendency to plasma albumin resulting in a meager portion of oral dose to reach the desired site. For avoiding the risk and cost from the high oral dose, localized delivery of LAPA is expected to obtain the same anticancer efficacy with much less dose of LAPA.

Hu et al. [116] designed another DDS to imitate the clinical administration of oral LAPA and injectable PTX. PTX-NPs and microparticles of LAPA (LAPA-MPs) were encapsulated in a thermosensitive injectable hydrogel made from Pluronic F127. The results of cytotoxicity assays revealed the most synergistic effect between LAPA and PTX against the HER2 and P-gp overexpressing BC cell lines. Furthermore, localized sustained drug release and promising combinational effects with very low dose of LAPA brought impressing anti-tumor effect *in vivo*. In addition, gel group showed less toxicity, which caused less body weight loss and less tissue injury. Daily oral administration of high dose of LAPA also resulted in more drug accumulation in some key tissues like liver, heart and lung. The drug concentration in tumors tissues was detected between gel and oral groups at different time point. PTX in both groups had a faster release than LAPA at first, and the high concentration maintained less than one week. However, the changing pattern of LAPA residue in tumor site was quite different between these two combinational groups. Although the dose of oral LAPA was very high, the amount of LAPA in oral group was very low at beginning, and increased slowly with time. While in gel group with very low dose of LAPA, the primary amount of LAPA was relatively high and kept at a steady level during the detection time.

3.7. Other nano-formulations

Agrawal et al. [117] designed HA-coated lapatinib nanocrystals via high-pressure homogenization technique. Hyaluronic acid (HA), a natural hydrophilic polysaccharide, not only extends the circulation time by evading RES-mediated clearance but also participates in tumor targeting. Cluster of differentiation 44 (CD44), a marker of a wide range of malignant cells, is a critical cell surface receptor for HA. [118,119] *In vivo* trials, these HA-coated nanocrystals showed enhanced endocytosis. It also reported that the higher uptake of LAPA encapsulated HA coated nanocrystals (LAPA-HA-NCs) resulted in higher apoptosis and disruption of mitochondrial membrane potential in MDA-MB-231 cells in concentration and time dependent manner. One of the reasons for apoptosis induction is the dysfunction of mitochondria which results due to the opening of mitochondrial permeability pore and releases apoptogenic factors causing loss of oxidative phosphorylation which result in cell death (Fig. 4). In this study, sufficient evidences were provided, which supported and demonstrated the magnificent tumor targeting capability of LAPA-HA-NCs against Triple-Negative Breast Cancer (TNBC). Overall, the administration of LAPA-HA-NCs can be an effective approach in the treatment of TNBC and its metastasis.

In a similar manner to liposomes, polymeric nanovesicles also can physically encapsulate hydrophilic agents in the aqueous cores and hydrophobic agents in the lamellar membranes. As shown in Fig. 5, Chen et al. [120] designed a smart pH- and redox-responsive polymeric nanovesi-

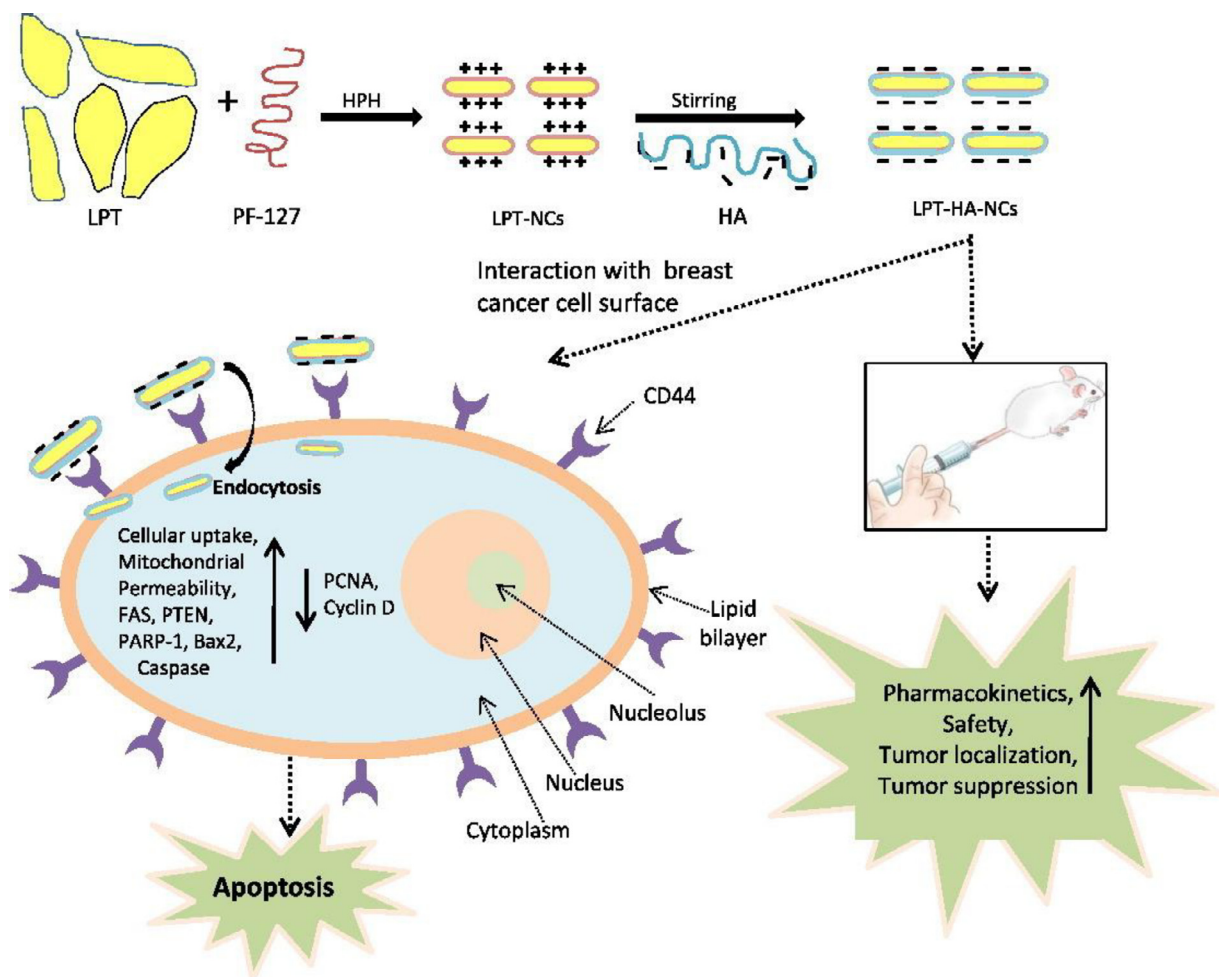


Fig. 4 – Enhanced delivery of LAPA in the form of HA coated nanocrystals formulation against triple negative breast cancer. External coating of HA actively targeted extracellular CD44 receptor thereby causing preferential accumulation of LAPA around the tumor cells. Intracellularly, LAPA led to programmed cell death, apoptosis, through the series of activation of apoptosis markers.

cle based on a copolymer of PEG and a polypeptide derivative, P(Asp(DBA-coMEA)-b-Phe. Lipophilia GEF and hydrophilic DOX can be simultaneously encapsulated in the shell and core of these nanovesicles via double-emulsion solvent evaporation method and the vesicles incorporating dual drugs displayed prominent pH/redox sensitivities to trigger the release of GEF and DOX inside cancer cells. This approach manifested a synergistic anticancer effect both *in vitro* and *in vivo*. Particularly, a remarkable therapeutic effect was observed *in vivo* in N2a cell line induced neuroblastoma model.

To overcome the limitations of both polymeric and lipid nanocarriers, Mandal et al. designed core-shell type lipid-polymer hybrid nanoparticles (LPNs) for the delivery of ETB [121]. In this innovative DDS, lipid membranes can improve permeation and cellular internalization, and the polymeric cores are able to control the drug release behavior. ETB-encapsulated LPNs (E-LPNs) were prepared by single-step sonication method as the polymeric core and phospholipid-shell structure consisting of 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy polyethyleneglycol]-2000] (DSPE-PEG2000) and hydrogenated

soy phosphatidylcholine (HSPC). The optimized E-LPNs were obtained with a mean size of about 170 nm, narrow PDI below 0.2, drug EE of about 66% and excellent storage stability. Cellular uptake and colony formation assays were conducted to evaluate the efficacy of E-LPNs in NSCLC cells. As compared with ETB solution, the results revealed enhanced cellular internalization and anticancer effects of E-LPNs. Therefore, LNP might be a potential tool for ETB delivery in cancer treatment.

Li and co-workers reported an innovative dual drug delivery platform based on disulfide-bridged quercetins (QSSQ) which was synthesized by modification of multiple phenolic hydroxyl groups on quercetin [122]. Some research has manifested that linking two hydrophobic monomers with disulfide bridge can facilitate the self-assembly of molecules into NPs. In this study, QSSQ successfully self-assembled into NPs and meanwhile encapsulated ETB during the process to form ETB-encapsulated NPs (E-QSSQ). As a natural product of flavonoids with diverse biological activities, quercetin exhibits antitumor effects through modulating several key elements located downstream of EGFR signaling pathways [123–125].

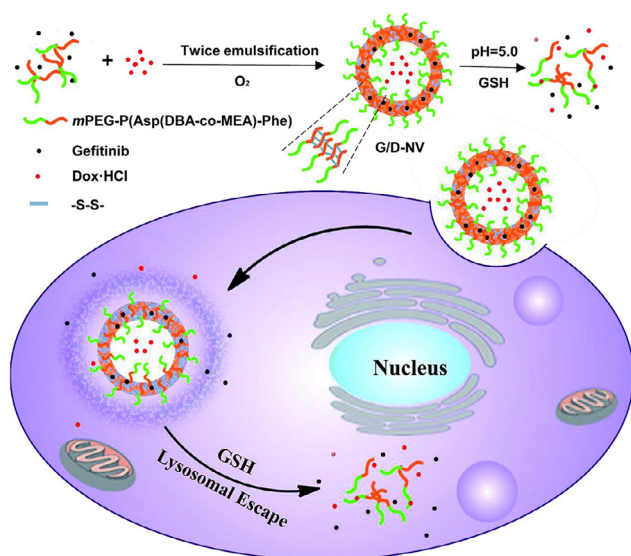


Fig. 5 – Illustrative preparation of nanovesicle as well as the dual sensitive release of DOX and GEF inside tumor cell.

Additionally, the overexpressed glutathione (GSH) in tumor tissue can trigger the release of erlotinib and quercetin via cleaving the disulfide bond in QSSQ. *In vitro*, a lower IC₅₀ value (4.1×10^{-6} M) and a higher percentage of apoptosis (28.5%–75.6%) against A549 cells for E-QSSQ than free ETB (8.7×10^{-6} M, 13.1%–58.0%), respectively. E-QSSQ, meanwhile, showed a high antitumor activity in a xenograft model of NSCLC *in vivo*. These results consistently corroborated that E-QSSQ was able to block the autophosphorylation at both upstream and downstream of EGFR signaling pathways, and therefore interrupt the EGFR signaling more potently, and eventually prevent the growth of EGFR-overexpressing tumor cells.

4. Conclusion and outlook

Over the past 20 years, great efforts have been made to design various inhibitors targeting EGFR family in cancer treatment. These unique small molecular inhibitors for EGFR have brought clinical benefits in various types of human malignancies.

However, the drawbacks related to the inherent property of these small molecule agents couldn't be ignored, including poor specificity, unexpected pharmacokinetics and biodistribution, which generally exert more serious damage to normal tissues, causing increased toxicity and side effects.

Supported by the rapid development of nanotechnology, EGFR-TKIs loaded nanocarriers show substantial effects on increasing solubility, prolonging systemic circulation, elevating accumulation in tumor site and reducing distribution in the normal tissues, which lead to significant enhancement on therapeutic efficacy. And more co-delivery systems were designed for simultaneously encapsulating multiple anticancer agents and controlling drug release in a predictable manner to maximize the synergistic effects. Though current results on EGFR-TKIs nanosized formulation to date seems hopeful in

preclinical researches, there are still many challenges that are required to be addressed in order to attain clinical potential.

First of all, the ability of nanocarriers to hold the cargoes and maintain the dosage ratio from the administration site to the final targets is also challenging. The difference in the encapsulation capacities of the nanocarriers results in inconsistent tendencies in premature leakage during the delivery process. Accordingly, for the future development of EGFR-TKIs DDS detecting the final drug concentration at the target site is a prerequisite. Secondly, the multifunctionality of nanocarriers ensure more efficient of targeting delivery, while it brings about complexity of material synthesis and difficulty of biodegradation. This suggests that the future material design should focus on multifunctionality, optimized synthesis, biodegradation and safety of primary/secondary metabolites etc. Thirdly, challenge for nanocarriers is targeting delivery EGFR-TKIs to a specific cell. The difficulty lies in the efficient recognition of a targeted cell among thousands of untargeted cells. To date, most of research efforts at present mainly focused on the delivery efficiency in one or two cell lines. However, the cellular delivery targeting single cell line in a mixture of different cell types is desired and should be pursued. Lastly, multifarious tumor cell-associated endogenous stimuli have already been applied for controlled drug release in a programmed manner. However, these stimuli, such as redox potential or acidity change due to individual differences. The most efficacious stimuli should be taken into account for clinical practice.

Conflicts of interest

The authors declare that there is no conflicts of interest.

Acknowledgments

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ajps.2019.06.001.

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